

## A Review of Nitrite and Chloride Chemistry: Interactions and Implications for Cured Meats

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Concerns for the health-related implications of sodium nitrite and sodium chloride in cured meats have lead to reductions in use of both ingredients and there is evidence of chemical interaction between nitrite and chloride in food systems which have implications for cured meats. This review considers the role of chloride in nitrite chemistry and extends this consideration to evaluation of potential changes in cured meat products. Microbiological control depends on nitrite reactivity and a chloride effect has been demonstrated in *Clostridium botulinum* spore outgrowth and toxin formation. Changes in flavour of cured meats or in development of cured colour are of lesser concern but may take place. More information on specific inhibitory mechanisms by nitrite is needed.

**Keywords:** Nitrite, chloride, nitrite-chloride interactions, cured meats, chloride catalysis, botulinal growth inhibition.

### 1. Introduction

Sodium chloride and sodium nitrite are the two ingredients in cured meat distinctive to this category of processed meat products, and are responsible for a major part of the colour, texture, flavour, safety, and storage stability characteristics unique to cured meat. Eliminating either salt or nitrite so alters product characteristics that the resultant meat mixture is no longer considered 'cured'. Consequently, both ingredients are absolutely necessary for the successful production of typical cured meat products.

Both sodium chloride and sodium (less commonly potassium) nitrite have generated independent concerns over their potential relationship to human health problems. Sodium chloride, added to cured meat at about 2.0%–2.5%, is a source of sodium in the diet and sodium has been implicated as a significant contributor to hypertension and related problems.<sup>1</sup> Sodium nitrite is added to meat at the parts-per-million level and therefore contributes very little sodium to total dietary intake. However, nitrite has been a concern in cured meat due to the formation of carcinogenic *N*-nitroso compounds. Work on the nitrite-nitroso compound problem has been underway for more than 25 years and has generated an immense amount of new information.<sup>2,3</sup> One result of this work has been a gradual reduction of nitrite levels in cured meat. The levels currently chosen depend upon the specific product being considered, but generally represent a compromise between the potential for formation of nitroso compounds at high nitrite levels and the potential for *C. botulinum* toxin formation at low nitrite levels. The best example of this compromise is the current USDA requirement for 120  $\mu\text{g g}^{-1}$  formulated nitrite (neither more nor less) in pumped bacon. This is less than that allowed for other cured products and reflects the specific problem of nitrosamine formation during the frying of bacon. Control of ingoing nitrite levels, use of reductants (ascorbate or erythorbate), and adherence to good manufacturing

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practices will substantially reduce the problem.<sup>4</sup> There is some concern for potential botulism problems should nitrite be reduced much below current levels.<sup>5,6</sup>

At the present time, reductions in sodium chloride usage in meat is a very active area of research.<sup>7-9</sup> Most research has centred on the organoleptic (flavour and texture) and microbiological aspects of sodium chloride, with the general objective of reducing the levels of sodium chloride in meat as much as possible.<sup>10,11</sup> It appears that a significant reduction can be accomplished, but the lower limits are not yet clear. Salt remains a major cure component for inhibiting *C. botulinum* outgrowth. Since nitrite levels have already been lowered in some products, an accompanying reduction of salt should be considered with care. Changing chloride levels could alter some or all of the several chemical reactions characteristic of nitrite during meat curing since chloride ions interact with nitrite.<sup>12,13</sup> The reactions of nitrite have been reviewed,<sup>14</sup> and have been shown to depend upon several variables inherent to meat mixtures. Nitrite/chloride interactions have not been so well addressed; the limited available information often appears contradictory.

This review considers nitrite chemistry from the aspect of potential chloride involvement with nitrite and discusses some subsequent implications for reduced sodium chloride levels in cured meat. For a proper perspective, it is important to understand first the general behaviour of the nitrite ion. In a dynamic system with multiple reaction pathways and infusion of reactants, i.e. oxygen from the atmosphere into cured meats, the relative amounts of various end products are functions less of individual equilibria, but more of relative velocities of the reactions. Both the absolute and relative amounts of end products can be affected by minor variations in reacting and interfering compounds. As a result, nitrite-chloride chemistry in meat systems is subject to considerable variation.

## 2. Chemistry of nitrite

### 2.1. Ions

#### 2.1.1. Nitrous acid reactions

The term nitrite is used generically to denote both the anion,  $\text{NO}_2^-$ , and the neutral nitrous acid  $\text{HNO}_2$ , but it is the latter which forms nitrosating compounds. The acid dissociation constant for the nitrous acid/nitrite equilibrium is  $3.98 \times 10^{-4}$  ( $\text{p}K_a = 3.4$ ); at the pH of meat (5.5–6.5) the concentration of the reactive acid is less than 1% of the total nitrite.<sup>15</sup> At *ca*  $2 \text{ mM NO}_2^-$  (150 ppm) and pH 5.8, the concentration of  $\text{HNO}_2$  is *ca*  $8 \mu\text{M}$  ( $600 \text{ ng g}^{-1}$ ). Even so, the very low nitrous acid concentrations generate some very reactive nitrosating species.<sup>16</sup>

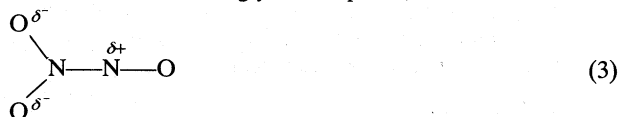
The first step in the reaction sequence beginning with nitrous acid is the generation of either a nitrosating species of the neutral radical nitric oxide (NO). The strongest nitrosating species are a form of a positively charged (electrophilic) nitrogen oxide, either in its simplest form the nitrosonium ion,  $\text{NO}^+$ , or as part of a larger molecule. In strong acid two species are formed, the nitrous acidium ( $\text{H}_2\text{NO}_2^+$ ) and nitrosonium ( $\text{NO}^+$ ) ions.



It is not definitely established that the nitrosonium ion exists free in solution; it probably exists as a part of the nitrous acidium ion, the positive charge being induced by charge transfer from the proton. Neither form exists at the pH of meat in appreciable quantities; the principal reactive species is the anhydride of nitrous acid, nitrogen trioxide.



The anhydride is stabilised in aqueous solution by resonance which results in a charge shift to form an electron-poor site ( $\delta^+$ ) in the molecule that is strongly electrophilic, and



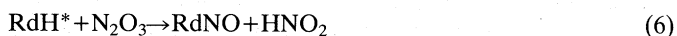
which will readily nitrosate nucleophilic sites to form nitroso compounds.<sup>17,18</sup>

### 2.1.2. Nitric oxide

Another important form of nitrite is nitric oxide (NO). The nitrogen is reduced by one electron from the nitrite-nitrogen and may be formed from dismutation or reducing reactions.



(one of the few termolecular reactions known)



(\*Rd—reductants, ascorbate, sulfhydryl groups, hydroquinones, etc.)



Reaction 4 takes place in strong acid and reaction 5 is relatively slow, leaving the sequence of reactions 6 and 7 as the major source of nitric oxide in meat, since the last is a rich source of reductants.

Nitric oxide is an electron-pair donor and forms very stable complexes with transition metals. The coordinate-covalent complexes of nitric oxide with the haem pigments of meat—nitrosylmyoglobin, nitrosylhaemoglobin, and dinitrosylhaemochrome—form the pink and red colours of cured meats.<sup>19,20</sup>

Nitric oxide is readily oxidised, which accounts in part for the instability of cured meat colour in air:



This reaction may account for the antioxidant properties of nitrite since it is very fast. In addition, nitric oxide is a radical chain terminator,<sup>21</sup> which would account for the effectiveness of nitrite in preventing lipid oxidation. The conversion of NO to NO<sup>+</sup> (or equivalent nitrosating species), which has been postulated to occur during bacon processing, may proceed through this type of recycling. The nitrogen dioxide (NO<sub>2</sub>) formed in reaction (8) will react further either to form nitrogen trioxide



or with water to form nitrous and nitric acids



These are both backwards reactions, regenerating previous reactants in the sequence (N<sub>2</sub>O<sub>3</sub>, HNO<sub>2</sub>). It will be noted that nitrate is produced in this recycling (reaction 10) and, since it is relatively unreactive, it acts as a sink to remove nitrite from the system. This constant recycling results in a semi-stable equilibrium of reactants, intermediates, and products.

While these reactions are the major characterised chemical reaction sequences, there are many other reactions that can and do take place. Even nitrous oxide (N<sub>2</sub>O, laughing gas) has been identified in the gases above curing mixes.

### 2.2. Reductant reactions

As noted previously, the concentration of nitrous acid is very low in meats, hence the nitrosation potential of nitrite is very low also. Under these conditions it requires a strong nucleophilic reagent to react with the nitrosating species at a rate sufficient to have a significant effect on the multiple reaction equilibrium. The most nucleophilic species in meats are the endogenous reductants of muscle cells as well as added ascorbate, and amine groups of the amino acids. Comparing data for the rates of nitrosation of amines<sup>22</sup> with rates of production of nitric oxide by reductants<sup>23–29</sup> it is found that reductants are one to ten thousand times as reactive as amines. The equilibrium is primarily controlled, therefore, by the reductant concentrations. Of course, stable nitrosation reaction products will act as sinks and buildup of these products will slowly

shift the equilibrium. Nitroso-reductants are unstable and do not accumulate, whereas *N*-nitroso compounds will accumulate, possibly even as high as millimolar quantities.<sup>30</sup> Meats as a food class have relatively high concentrations of reductants, including reduced nicotinamide adenine dinucleotide, quinoid-type and other reducing coenzymes, sulphhydryl groups and, of course, added ascorbic and/or erythorbic acid.<sup>23-25</sup>

The reduction itself is an unusual reaction for it is a one-electron reduction step from the nitrite-nitrogen to the nitric oxide-nitrogen. Nitric oxide is an odd-electron compound and the reaction would be expected to produce a reductant radical, but such is not the case, at least not with ascorbate.<sup>29</sup> A dinitrosylascorbate intermediate was proposed for the reduction to account for the lack of formation of the ascorbyl radical. The nitroso-reductant intermediate (RdNO) of reaction 6 and others have been found to be semi-stable,<sup>24,26,29</sup> and therefore probably contribute to decreased nitrosation capacity by removing  $N_2O_3$  from nitrosation reactions. The semi-stable intermediates however, could also represent a reservoir of nitrite for later regeneration of nitrosating species. This may partially explain why ascorbate has not been 100% effective in blocking nitroso compound formation, especially at high ascorbate levels.<sup>31</sup> Reductants are generally polar compounds, especially ascorbic/erythorbic acids, and are poorly soluble in nonpolar systems. Since adipose tissue is a major source of nitrosamines, low reductant solubility would also contribute to low effectiveness in reducing nitrosamine formation. Nitric oxide generated by reductants may have a special function as a lipid antioxidant since it is soluble in nonpolar systems.

### 2.3. Multiple reactants

Other reactants have been suggested, including unsaturated fatty acids, tyrosine, amide linkages in proteins, tryptophane, amino sugars, and browning reaction compounds. A review of these reactions has been made by Cassens *et al.*<sup>14</sup> Suffice it to say that while many of these reactions are minor when compared with reductant reaction, in the sum they could account for a significant amount of nitrite bound in a variety of forms. Most of such bound nitrite is available as free nitrite since the nitroso derivatives are relatively unstable compounds which, with the exception of nitroso-reductants, decompose to yield the initial products. The absolute and relative amounts of these compounds are not fixed but will vary with acidity, water activity, oxygen concentration, temperature, etc.

### 2.4. Temperature

In a multiple reaction system, if the activation energies were all the same, the same mix of reactants, intermediates, and products would prevail at all temperatures. This of course is not the case. The activation energies (heats of activation) of the formation of different nitrosating species are not all the same, nor are the activation energies of the subsequent nitrosation reactions.<sup>25</sup> Where a reaction rate is controlled by an equilibrium concentration, the rate will vary as the equilibrium constant changes with temperature.<sup>32</sup> The consequence is therefore that the mixture of products will vary with temperature in a multiple substrate system. Furthermore, the activation energies of the nitrosation reactions vary with pH, sometimes as a result of the formation of two or more products from the same substrate, thus different product mixtures will be found at different pH values even at the same temperature. Finally, varying the time at which heat is applied during processing will produce different compositions in the mix, since the reaction conditions at the time at which heating is initiated will vary.

### 2.5. Transnitrosation

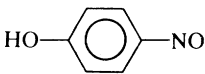
In a complex reacting mixture it is technically possible that a given compound would nitrosate more readily than others, yet not be as stable as the rest. In this case, the possibility exists that the first compound will preferentially form a nitroso compound that would then transfer the nitroso group to another reactant whose nitroso derivative was more stable, the process being known as transnitrosation. A modified form of transnitrosation has been demonstrated,<sup>33,34</sup> but

the transnitrosating species were formed under one set of conditions and mixed with the receptor compounds under another set of conditions. Transnitrosation between compounds in the same mixture is a more difficult process to prove and requires the demonstration that the recipient compound would not have otherwise nitrosated at the same rate without the presence of the presumed transnitrosating species.

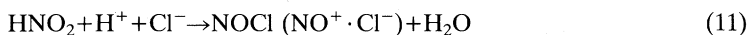
### 3. Role of chloride

Yet another factor adding to the complexity of nitrite chemistry is the ability of electronegative anions to form resonance stabilised, charge separated molecules from nitrous acid, thereby creating other nitrosating species (Table 1). In general, they are more reactive than nitrogen trioxide ( $\text{N}_2\text{O}_3$ ) and less reactive than the nitrous acidium ion ( $\text{H}_2^+\text{NO}_2$ ). The ordering in Table 1 is a composite derived from the ordering of several sequences in the literature.<sup>35-40</sup> The first two

Table 1. Relative reactivities of various nitrosating species

Species	Source
Fastest	
$\text{NO}^+$ , $\text{H}_2\text{NO}_2^+$	Protonation of $\text{HNO}_2$ , found only in strong acid solutions
$\text{NOSCN}$	From saliva
	From smoke which has many other phenolic compounds
$\text{NOCl}$	From gastric juice, curing salts
$\text{N}_2\text{O}_4$	Found in air
$\text{N}_2\text{O}_3$	Nitrous acid anhydride
Nitroso derivatives of citrate, acetate, sulphate, phosphate	Cure ingredients, weakly reactive under certain conditions
Slowest	

forms are produced only in strong acid and therefore are not of concern for meat reactions since meat and/or products have pH values ranging from  $\sim 4.5$ – $6.5$ . The gradations toward weaker nitrosating species occur because of the decreasing electronegativity of that part of the molecule attached to the  $\text{—NO}$ . The most effective anion is the thiocyanate ion, a pseudohalogen, which is of interest since it occurs in saliva.<sup>35</sup> The next most effective anions are the halogens, of which chloride is the ion of interest in meat. Nitrous acid and hydrochloric acid will generate nitrosyl chloride ( $\text{NOCl}$ ).



Formation of nitrosyl chloride as opposed to formation of nitrogen trioxide has a number of important consequences.

#### 3.1. Increased rate of reaction

An increased reaction rate may be beneficial in providing more rapid formation of cured colour or the suppression of bacterial growth in cured meats. However, since nitric oxide, the nitrosonium ion, and other reduced nitrogen oxides may be oxidised back to nitrite or nitrate, faster reduction also means faster recycling in the presence of air, hence rapid depletion of residual reductants.

#### 3.2. Difference in specificity

Relative rates of reaction of the various nitrosating species will vary depending on the compounds nitrosated. If nitrosyl chloride reacts with compound A twice as fast as does nitrogen

trioxide, it does not follow that the former will react twice as fast with compound B as will the latter. For example, while chloride accelerates nitrosyl hemochrome formation and the Griess reaction used to determine nitrite,<sup>41</sup> it appears to decrease the formation of nitrosopyrrolidine.<sup>42</sup> Thiocyanate ion had no effect on the nitrosation of amides, but accelerated nitrosation of amines; citrate and tartrate anions accelerated nitrosamide formation, but either decelerated or had no effect on nitrosamine formation.<sup>40</sup> Compounds produced will vary with the form of the nitrosating species. While  $N_2O_3$  forms the corresponding nitrosamines from dibenzylamine or trimethylamine, the major products with NOCl are the corresponding chlorides.<sup>43</sup> In the Griess reaction, chloride also causes different amounts of pigment to be produced, depending on the reagent combination used.<sup>41</sup>

### 3.3. pH effect

The dependence of nitrosation on acidity is different for NOCl compared with  $N_2O_3$ . The rate of amine nitrosation by  $N_2O_3$  decreases rapidly with pH below the  $pK_a$  of nitrous acid due to protonation of the amine.<sup>36</sup> The nitrous acidium ion reaction and the halide catalysed reactions do not show this decrease as greatly, probably because being more powerful nitrosating species they are less sensitive to the protonation of the amine; that is, they react almost as well with the acid form as with the free amine. The reactivity of nitrosyl chloride is high; gaseous NOCl may be used for quantitative synthesis of nitroso compounds,<sup>44-46</sup> and nitrosation of amines can occur under milder conditions when NOCl is present than when the nitrosating species is  $N_2O_3$ . On the other hand, NOCl is not as effective at higher pH values as some other nitrosating species. Thiocyanate and iodide accelerate nitrosamine formation fairly uniformly over a wide range of pH,<sup>36</sup> but chloride inhibited both nitrosopyrrolidine formation in cured meat systems<sup>42</sup> and nitrosoproline formation at the pH values of cured meats.<sup>47</sup> Other reports have indicated that salt did not affect formation of nitrosodimethylamine in a model system.<sup>31</sup> The effect of salt may be difficult to predict because of the opposing effects of high ionic strength and catalysis by chloride.<sup>17</sup> At the time of writing, it appears that sodium chloride does not increase nitrosamine formation in cured meats and may provide some inhibition, but the mechanism is not known. In this regard, it should be pointed out that at the pH of meat the rates of the nitrosation reactions of all species are considerably reduced, so that product yields become very small, even with catalysis. Reactant concentrations are usually molar or millimolar, but intermediate and product concentrations are much lower, millimolar or nanomolar. Product concentrations thus range from  $10^{-3}$  of the initial reactant concentrations at best to  $10^{-9}$  at worst. While we assume that the laws of kinetics and mass action are not suspended under these conditions, definitive statements concerning mechanisms at very low product formation levels are difficult to make with certainty.

### 3.4. Nitrite and salt concentration effect

The relative concentrations of  $N_2O_3$  and NOCl are dependent on the nitrous acid and chloride concentrations. The formation of  $N_2O_3$  is bimolecular in nitrous acid, whereas the formation of NOCl is monomolecular. In a system where both species are formed, a doubling of the nitrous acid concentration will result in a fourfold concentration increase in  $N_2O_3$ , but only twice as much NOCl. Since  $N_2O_3$  and NOCl differ in their reactivity in meat systems, the relative amounts of products will also be different at the two different concentrations. Salt also changes the tertiary structure of the proteins, which would affect the reactivity of the reactive groups in the protein.

### 3.5. Temperature

As noted earlier, the time at which the temperature is changed during meat processing is likely to cause a difference between salt and no-salt products, since the concentrations of intermediates and products of the nitrosation reactions will have a different time dependence.

In view of the foregoing observations, it is clear that the nitrosating ability or potential of a given compound must be defined in terms of both the compounds being nitrosated and the

environment in which the reactions take place. The ordering in Table 1 is generalised, therefore, and does not hold for all reactions under all conditions.

#### 4. Effects of chloride in model system

##### 4.1. Nitrite determination

Changes in measurable (residual) nitrite or nitrosyl haem pigments may result from changes in chloride levels. These changes can be measured reasonably well, but the few studies performed in this area have reported contradictory results. Early studies of nitrite in heated, buffered, aqueous solutions reported that increasing sodium chloride levels resulted in greater residual nitrite concentration,<sup>48</sup> and other model system studies have found similar results.<sup>49</sup> Studies using model meat systems have reported either no effect by chloride,<sup>50</sup> or decreased measurable nitrite as sodium chloride increased.<sup>51</sup> One of the problems with these studies is, as noted previously, that chloride catalysis of diazo pigment formation during nitrite determination shows a reagent specificity.<sup>12</sup> The amounts of Griess pigment formed at different salt levels depended on the reagent combination used. Apparent nitrite values were increased by chloride levels when sulphanilic acid was used as the nitrosatable species,<sup>12,52</sup> but when sulphanilamide was used chloride had no effect.<sup>12</sup> Thus, some of the earlier attempts to measure residual nitrite may have included some error due to chloride affecting the analytical method. Unfortunately, in many studies reported it is not always readily apparent which reagents were used. Chloride addition sometimes causes a pH shift in meat systems and at least part of the change in residual nitrite that is attributed to chloride should more properly be credited to changes in acidity.<sup>51</sup>

While results from the Griess reagent studies indicate formation of NOCl at a somewhat higher pH than previously reported, the system is still considerably more acidic than most foods, and the question of NOCl formation at pH 5.5 or higher still remains. Comparisons of the effect of chloride and reductants on measurable nitrite in a pH 5.6 meat slurry have recently been made,<sup>12,13</sup> using several nitrite determination methods. Table 2 shows results from the Griess method, chemiluminescent detection and differential pulse polarography used for cooked meat slurries. Although there is some difference as a result of analytical methodology, a relative reduction of measurable nitrite with increasing sodium chloride is almost universally demons-

**Table 2.** Effect of NaCl and reductant on measurable nitrite in a cooked meat slurry (initial nitrite=138–145 ppm)

NaCl (mm)	Reductant	Nitrite (as % of initial)			
		Gr <sup>a</sup>	GR <sup>b</sup>	CLD <sup>b</sup>	DPP <sup>b</sup>
0	0	91	81	76	82
0.5	0	91	—	—	—
1.0	0	84	72	80	75
0	3 mm ascorbate	84	56	78	50
0.5	3 mm ascorbate	68	—	—	—
1.0	3 mm ascorbate	65	38	47	8
0	6 mm ascorbate	73	—	—	—
0.5	6 mm ascorbate	59	—	—	—
1.0	6 mm ascorbate	49	—	—	—
0	12 mm ascorbate	55	24	52	20
0.5	12 mm ascorbate	44	—	—	—
1.0	12 mm ascorbate	35	17	33	3
0	20 mm cysteine	—	57	58	56
1.0	20 mm cysteine	—	45	50	44

<sup>a</sup>From Fox *et al.*<sup>11</sup> 1981; Gr=Griess reagent.

<sup>b</sup>From Fox *et al.*<sup>12</sup> 1982; GR=Griess reagent, CLD=chemiluminescent detection, DPP=differential pulse polarography.

trated. An analysis of variance showed a significant three-way interaction between salt, reductant, and heating; the authors attributed the differences in loss of nitrite to formation of NOCl with increased activity. An accompanying model system which contained only buffer, nitrite, ascorbate, and salt did not show the chloride effect observed in the meat slurry. The chloride/ascorbate interaction in the meat systems therefore must have involved tissue components. Since, as we have noted, the major (fastest) nitrosation reaction is with ascorbate, the tissue component reactions must involve secondary reactions with nitrosoascorbate intermediates or reaction products thereof.

#### 4.2. Cured meat pigment

In addition to measurable nitrite, another test of formation of NOCl with increased nitrosating ability would be enhanced production of nitrosyl haem pigments due to increased production of the nitroso-reductant intermediate. In this case, the haem pigment is the 'acceptor' molecule for nitric oxide from the nitroso-reductant. If significant NOCl is formed, increased nitrosating activity should result in increased nitric oxide and subsequent nitrosyl haem formation. Studies at relatively high salt concentrations have reported that final colour intensity of the nitrosyl haem was decreased by increasing salt.<sup>54</sup> At high salt levels, however, salt concentrations may result in partial denaturation of the globin and other proteins which will increase the opacity of the cell tissue, thereby reducing the molar absorbance of the pigment *in situ*. A model system study showed that added sodium chloride resulted in increased nitrosyl haem pigment formation,<sup>49</sup> and a fivefold increase in the reaction rate constant has been noted for the formation of nitric oxide in 3 M (~24%) NaCl as compared to no addition of NaCl.<sup>55</sup> The latter study also reported a gross morphological change in the interfibrillar spaces of cured beef muscle that was not caused by either sodium chloride or nitrite alone. The authors attributed this difference to formation of NOCl. Reaction rate constants for formation of nitric oxide from nitrite-reductant mixtures have been found to increase linearly with increasing NaCl from 0–4% (J. G. Sebranek, unpublished data). Other chloride salts gave similar results.

This same study also showed the effect of multiple reactants on the final equilibrium. The catalytic effect of chloride on nitrosyl haem pigment formation was evident under nitrogen, but mixtures with all levels of chloride reached the same final pigment concentration. When the system was studied in air, oxygen diffusion into the system created competition for the generated nitric oxide and those samples that generated nitric oxide more rapidly (higher chloride) resulted in greater total nitrosyl pigment. Thus, as noted earlier, the overall effect of chloride levels on nitrite reactions depends not only on the catalytic effect, but also on competing reactions and infusion of reactants (O<sub>2</sub>) in the system.

### 5. Chloride catalysis in cured meats

The potential influence of chloride on nitrite related reactions has many implications for cured meat. For example, assume NOCl reacts faster with reductants than does N<sub>2</sub>O<sub>3</sub>. The concentration of NOCl will be reduced proportionally more than will be the concentration of N<sub>2</sub>O<sub>3</sub>, with more nitrite in the relatively unreactive forms of nitric oxide, nitroso-reductants and nitrosyl haem pigments. Since chemical potential is the product of activity times concentration, the total nitrosating potential of the lower NOCl concentration could well be less than that of the higher N<sub>2</sub>O<sub>3</sub> concentration, even though the former is a more powerful nitrosating species than the latter. A lower nitrosating potential for *N*-nitrosation coupled with a reservoir of nitric oxide, equivalent to nitrite, is, *a priori*, a desirable state for cured meats. Of course, since nitrosating species are reactant-specific, the real situation might be the reverse, with N<sub>2</sub>O<sub>3</sub> the more powerful nitrosating species for reductants.

#### 5.1. Colour

Nitrite research has established that colour development in cured meat can be adequately accomplished with 25–50 mg kg<sup>-1</sup> nitrite.<sup>56–59</sup> This may vary with different products and storage



conditions, but generally, nitrite added to meat for curing ( $100\text{--}150\text{ mg kg}^{-1}$ ) is in excess of that needed for colour development alone. In a study of pigment formation in frankfurters, the pigment concentration was a function of a number of variables, including amount of oxygen present, temperature of processing and added reductant.<sup>60</sup> Since the per cent conversion was an equilibrium process with less than 100% conversion of the amount of pigment available, the amount of pigment was a function of the amount of residual nitrite and reductants available for reaction. These amounts are determined by the amount of oxygen present and the rate of the nitrite reduction-oxidation cycle. Faster turnover would mean lower reactant concentration, and lower pigment concentrations of lesser stability as noted earlier. Ascorbate and cysteine reduce nitrite at different rates, but no significant difference was observed in the quantity or stability of the pigments of frankfurters produced with the two reductants.<sup>60</sup> No study has been made of the effect of reduced chloride concentrations on the nitrite cycling rate and/or colour formation and stability.

## 5.2. Flavour

The nitrite normally used for curing may be more than that needed for cured flavour development,<sup>61-65</sup> but this function of nitrite in cured meat has not been associated with any specific flavour components. Sodium chloride alone is a very strong flavour component in cured meat<sup>66</sup> and affects flavour both in production of other flavour components and enhancement of the taste, although the two effects would be difficult to separate. Furthermore, salt alone is a pro-oxidant, leading to increased fat oxidation and rancidity.<sup>67-70</sup> It has been claimed that salt is the major factor responsible for cured meat flavour, rather than nitrite or the absence of lipid oxidation.<sup>71</sup> Nevertheless, salt pork and bacon have quite different flavours, clearly indicating nitrosation reactions in the latter. Long-term flavour retention of cured meat is also dependent upon nitrite for suppression of rancid flavours,<sup>72-74</sup> an effect which can be observed at nitrite levels as low as  $50\text{ mg kg}^{-1}$ .<sup>75</sup> This function of nitrite is accomplished in part by interaction of nitric oxide with iron compounds,<sup>76</sup> by reaction of oxygen with nitrogen oxide, by formation of a specific anti-oxidant from polar lipids,<sup>77</sup> and by radical chain termination. Chloride catalysis of nitric oxide formation would initiate inhibitory processes at an earlier phase of the processing procedure, and counteract the pro-oxidant effect of chloride alone.

## 5.3. Transnitrosation: sulphhydryl compounds

Nitrosothiols such as *S*-nitrosocysteine have also been reported to contribute antioxidant activity when synthesised and added to food systems.<sup>78,79</sup> Because they are inherently unstable, nitrosothiols have also been considered from the standpoint of transnitrosation or transfer of the nitroso group to another compound such as a secondary amine.<sup>80,81</sup> Catalysis of transnitrosation reactions by chloride in organic solvents can occur but it depends upon the nucleophilic compound involved.<sup>82</sup> There is little information concerning potential chloride catalysis of transnitrosation in food systems. In a system with both cysteine and ascorbate the latter is more electrophilic and would be nitrosated more readily, making nitrosothiol formation less likely. As a result, the role of nitrosothiols in nitrosation reactions becomes difficult to assess and whether they help suppress rancidity is not entirely clear.

## 5.4. Botulinum growth inhibition

Current research includes efforts to clarify the inhibitory mechanism(s) by which nitrite inhibits *C. botulinum*. The possible mechanisms include:<sup>83,84</sup> (1) formation of an inhibitory substance from nitrite and other meat components, (2) nitrite (or intermediates) acting as an oxidant or reductant on intracellular enzymes or nucleic acids, (3) restriction of iron (or other metals essential to *C. botulinum*) by nitrite thereby interfering with the organism's metabolism and/or biological repair mechanisms, and (4) reaction of nitrite with cell membranes to limit metabolic exchanges or substrate transport. More than one mechanism is likely in a complex system like meat.<sup>84</sup> For example, metal sequestering agents such as EDTA enhanced the inhibitory activity of nitrite while excess iron caused a decreased inhibition.<sup>86-88</sup> The suggestion has been offered

that nitrite, probably via nitric oxide, reacts with iron of ferredoxin in germinated cells.<sup>89</sup> Inhibition of *Clostridium sporogenes* by reaction of nitric oxide with the nonhaem iron of pyruvate:ferredoxin oxidoreductase has been reported.<sup>90</sup> Destruction of the iron-sulphur 'cluster' on proteins of vegetative cells of *C. botulinum* by formation of iron-nitric oxide complexes from added nitrite has recently been reported.<sup>91</sup> Another suggestion is the conversion of extracellular iron, essential to *C. botulinum*, to an unavailable form after reaction with nitric oxide.<sup>86</sup> Ascorbate also plays a role either as a metal chelator,<sup>91,92</sup> or as a means of generating more nitric oxide in the system. These mechanisms appear to be dependent upon nitric oxide and would be influenced by factors (such as chloride or acid catalysis) that control the rate or extent of nitric oxide formation.

In contrast to reactions involving nitric oxide, other researchers have suggested that microbial inhibition may derive from nitrosation reactions which produce a specific extracellular inhibitor,<sup>93</sup> or disrupt critical cysteine-containing enzymes.<sup>94,95</sup> However, other investigators have reported that bacterial inhibition is accomplished in meat without participation of the sulphhydryl groups.<sup>96</sup> Nitrosation reactions could result in a number of different compounds with inhibitory properties. Most likely among these are reaction products from amines, haem iron, unsaturated lipids, and thiols.<sup>83</sup> Interestingly enough, *N*-nitrosamines have been found to be non-inhibitory.<sup>97</sup>

Bacterial cell wall constituents may also react with nitrite and result in alteration of substrate uptake.<sup>94</sup> This potential reaction has not been studied in depth and there is, therefore, little indication of how significant it may be, though other known inhibitors such as the parabens and sorbate effectively inhibit substrate transport into cells.<sup>98</sup>

While the proposed nitrite inhibitory mechanisms are many and complex, that of sodium chloride alone is simple; namely it lowers the water activity. The concentration of added nitrite is of course much too low to have any effect on the water activity. When chloride and nitrite were used together however, there was a very pronounced interaction in their effect on botulinal spore outgrowth and toxin production. Roberts and Ingram<sup>99</sup> found that with the least sensitive strain of *C. botulinum* (type B), neither 6% NaCl nor 300 mg kg NaNO<sub>2</sub> alone inhibited spore growth at pH 5.8 and 35°C. Yet combinations of the two reagents below these concentrations were effective in inhibiting outgrowth, for example, 3% NaCl and 200 mg kg<sup>-1</sup> NaNO<sub>2</sub>, clearly indicating an interaction of the two reagents. Their results are perplexing. At the salt and nitrite levels usually employed in commercial practice, ca 2-3% NaCl and 100-200 mg kg<sup>-1</sup> NaNO<sub>2</sub>, they found little or no inhibition of either outgrowth or toxin formation from types A and B *C. botulinum*.<sup>6, 100, 101</sup>

Furthermore, none of the salt or nitrite levels tested inhibited toxin formation at 25°C. It is not immediately clear why commercial practice is successful in preventing botulinal poisoning, but in view of what appears to be a rather tenuous situation, and the fact that salt and nitrite interact to prevent outgrowth and toxin formation, the reduction of either or both salts in cured meats must not be undertaken without careful study of the consequences. The consequences may be considerable in view of the fact that the formation of nitrosyl chloride falls off faster as the chloride concentration decreases; that is, the amount of NOCl formed at 1% NaCl is much less than half that formed at 2% NaCl. The amount of the nitrosating species becomes proportionally much less as the concentrations of either salt or nitrite is lowered.

## 6. Conclusions

In the final analysis, chloride can significantly catalyse nitrite reactions under conditions occurring in meat. Reduced sodium chloride levels may result in decreased nitrosation of various substrates or may result in a decreased rate of nitric oxide production from nitrosation of reductants. The chemistry of nitrite is complex and becomes even more so in a meat system; the full extent of the significance of chloride reduction for nitrite reactions remains to be determined. It seems that cured flavour or pigment formation would be minimally affected since the nitrite level currently used is somewhat in excess of the minimum needed for these characteristics. The antioxidant function of nitrite might be adversely affected by changes in chloride level but the

antioxidant behaviour of nitrite has not been sufficiently quantitated to allow a good assessment. Control of *C. botulinum* by nitrite may be the area most sensitive to changes in the system since the minimum nitrite needed is likely more than that being placed on the system if sodium chloride, with its dual inhibitory contribution, is reduced. Considering that botulinum contamination in the world at large is sporadic and the inhibitory mechanism of nitrite is, at best, poorly understood, it becomes extremely difficult to estimate any 'critical' combination of salt and nitrite for assured safety. Certainly, the case against nitrite and salt in cured meats becomes moot with the first incidence of botulism from a low salt/nitrite product. A critical need at the present time is a better understanding of the nitrite reactions which inhibit *C. botulinum* growth and toxin formation. Until that information is provided, a reduction of sodium chloride in cured meat may have consequences that are not yet apparent and should be both carefully and thoughtfully evaluated.

## References

1. Pearson, A. M.; Wolzak, A. M. Salt—its use in animal products—a human health dilemma. *J. Animal Sci.* 1982, **54**, 1263–1277.
2. National Academy of Sciences. In: *The Health Effects of Nitrite and N-nitroso Compounds Part I* National Academy Press. Washington, DC, 1981.
3. Magee, P. N.; Barnes, J. M. The production of malignant primary hepatic tumours in the rats by feeding dimethylnitrosamine. *Br. J. Cancer* 1965, **10**, 114–122.
4. Dudley, R. How to keep your bacon out of trouble. *Meat Ind.* 1979, **26**, 24–32.
5. Hauschild, A. H. W. Assessment of botulism hazards from cured meat products. *Food Technol.* 1982, **36**, 95–104.
6. Roberts, T. A.; Gibson, A. M.; Robinson, A. Factors controlling the growth of *Clostridium botulinum* Types A and B in pasteurized, cured meats. II. Growth in pork slurries prepared from 'high' pH meat (range 6.3–6.8). *J. Food Technol.* 1981, **16**, 267–281.
7. Terrell, R. N.; Olson, D. G. Chloride salts and processed meats: properties, sources, and mechanisms of action, labeling. In *Proc. Meat Ind. Res. Conf.* 1981, pp. 67–94.
8. Sebranek, J. G.; Olson, D. G.; Whiting, R. C.; Benedict, R. C.; Rust, R. E.; Kraft, A. A.; Woychik, J. H. Physiological role of dietary sodium in human health and implications of sodium reduction in muscle foods. *Food Technol.* 1983, **37**, 51–59.
9. Terrell, R. N. Reduction of sodium in processed meat. *Food Technol.* 1983, **37**, 66–72.
10. Seman, P. L.; Olson, D. G.; Mandigo, R. W. Effect of reduction on bologna characteristics and acceptability. *J. Food Sci.* 1980, **45**, 1116–1121.
11. Whiting, R. C.; Jenkins, R. K. Partial substitution of sodium chloride by potassium chloride in frankfurter formulations. *J. Food Qual.* 1981, **4**, 259–269.
12. Fox, J. B., Jr.; Zell, T. E.; Wasserman, A. E. Interaction between sample preparation techniques and colorimetric reagents in nitrite analysis in meat. *J. Assoc. Off. Analyt. Chem.* 1981, **64**, 1397–1402.
13. Fox, J. B., Jr.; Doerr, R. C.; Lakritz, L. Interaction between sample preparation techniques and three methods of nitrite determination. *J. Assoc. Off. Analyt. Chem.* 1982, **65**, 690–695.
14. Cassens, R. G.; Greaser, M. L.; Ito, T.; Lee, M. Reaction of nitrite in meat. *Food Technol.* 1979, **33**, 46–57.
15. Challis, B. C.; Butler, A. R. In: *The Chemistry of the Amino Group* (Patai, S., Ed.) Interscience Publishers. New York, USA, pp. 306–310.
16. Bonnett, R.; Martin, R. A. Interaction of nitrite with haems and related compounds. *IARC* 1976, **14**, 487–492.
17. Boyland, E. The effect of some nitrite ions of physiological interest on nitrosamine synthesis. *IARC* 1972, **3**, 124–129.
18. Challis, B. C.; Kyrotopoulos, S. A. The chemistry of nitrosocompounds. II. Nitrosation of amines by the two phase interaction of amines in solution with gaseous oxides of nitrogen. *J. Chem. Soc. Perkin I* 1979, 299–304.
19. Fox, J. B., Jr. The chemistry of meat pigments. *J. Agric. Food Chem.* 1966, **14**, 207–210.
20. Giddings, G. G. The basis of color in muscle foods. *J. Food Sci.* 1977, **42**, 288–294.
21. De la Mare, H. E.; Vaughan, W. E. Chemistry of organic free radicals in the vapor phase. I. Detection and reaction of the free alkyl radicals. *J. Chem. Educ.* 1957, **34**, 10–14.
22. Kurosky, A.; Hofmann, T. Kinetics of the reaction of nitrous acid with model compounds and proteins, and the conformational state of *n*-terminal groups in the chymotrypsin family. *Can. J. Biochem.* 1972, **50**, 1282–1296.
23. Fox, J. B., Jr.; Nicholas, R. A. Nitrite in meat. Effect of various compounds on loss of nitrite. *J. Agric. Food Chem.* 1974, **22**, 302–306.
24. Fox, J. B., Jr.; Thomson, J. S. Formation of bovine nitrosylmyoglobin. I. pH 4.5–6.5. *Biochem.* 1963, **2**, 465–476.
25. Fox, J. B., Jr.; Ackerman, S. A. Formation of nitric oxide myoglobin: mechanisms of the reaction with various reductants. *J. Food Sci.* 1968, **33**, 364–370.
26. Dahn, H.; Loewe, L. Über die oxydation von ascorbinsäure durch salpetrige säure. Teil IV. Die reaktion nullter ordnung. *Helv. Chim. Acta* 1960, **43**, 310–317.
27. Dahn, H.; Loewe, L.; Bunton, C. A. Über die oxydation von ascorbinsäure durch salpetrige säure. Teil III. Kinetik im gebiet der Taylor schen gleichung. *Helv. Chim. Acta* 1960, **43**, 303–310.

28. Dahn, H.; Loewe, L.; Bunton, C. A. Über die oxydation von ascorbinsäure durch salpetrige säure. Teil VI. Übersicht und diskussion der ergebnisse. *Helv. Chim. Acta* 1960, **43**, 320-333.
29. Fox, J. B., Jr.; Fiddler, R. N.; Wasserman, A. E. Initial reaction intermediates in the oxidation of ascorbic acid by nitrous acid. *J. Food Protect.* 1981, **44**, 28-32.
30. Massey, R. C.; Key, P. E.; McWeeney, D. J.; Knowles, M. E. The application of a chemical denitrosation and chemiluminescence detection procedure for estimation of the apparent concentration of total *n*-nitroso compounds in foods and beverages. *Food Add. & Contam.* 1984, **1**, 11-16.
31. Fiddler, W.; Pensabene, J. W.; Kushnir, I.; Piotrowski, E. G. Effect of frankfurter cure ingredients on *N*-nitrosodimethylamine formation in a model system. *J. Food Sci.* 1973, **38**, 714-715.
32. Turney, T. A.; Wright, G. A. Nitrous acid and nitrosation. *Chem. Rev.* 1959, **59**, 497-513.
33. Dennis, M. J.; Davis, R.; McWeeney, D. J. The transnitrosation of secondary amines by *S*-nitrosocysteine in relation to *N*-nitrosamine formation in cured meat. *J. Sci. Food Agric.* 1979, **30**, 639-645.
34. Dennis, M. J.; Davis, R.; McWeeney, D. J. The transnitrosation of *N*-methylaniline by a protein bound nitrite model system in relation to *N*-nitrosamine formation in cured meat. *J. Sci. Food Agric.* 1980, **31**, 1195-1200.
35. Boyland, E.; Nice, E.; Williams, K. The catalysis of nitrosation by thiocyanate from saliva. *Food. Cosmet. Toxicol.* 1971, **9**, 639-643.
36. Fan, T.-Y.; Tannenbaum, S. R. Factors influencing the rate of formation of nitrosomorpholine and nitrite: acceleration by thiocyanate and other anions. *J. Agric. Food Chem.* 1973, **21**, 237-240.
37. Fridman, A. L.; Mukhametshin, F. M.; Novikov, S. S. Advances in the chemistry of aliphatic *N*-nitrosamines. *Russ. Chem. Rev.* 1971, **40**, 34-50.
38. Austin, A. T. Nitrosation in organic chemistry. *Sci. Prog.* 1961, **49**, 619-640.
39. Hughes, E. D.; Ingold, C. K.; Ridd, J. H. Nitrosation, diazotisation and deamination. I. Principles, background and method for the kinetic study of diazotisation. *J. Chem. Soc.* 1958, 58-65.
40. Yamamoto, M.; Yamada, T.; Tanimura, A. Studies on the formation of nitrosamines. V. The effects of citrate, tartrate, and thiocyanate on the rates of nitrosation. *J. Food Hyg. Soc.* 1976, **17**, 363-368.
41. Fox, J. B., Jr.; Kinetics and mechanisms of the Griess reaction. *Anal. Chem.* 1979, **51**, 1493-1502.
42. Theiler, R. F.; Sato, K.; Aspelund, T. G.; Miller, A. F. Model system studies on *N*-nitrosamine formation in cured meats: the effect of curing solution ingredients. *J. Food Sci.* 1981, **46**, 996-998.
43. Smith, P. A. S.; Loeppky, R. N. Nitrosative cleavage of tertiary amines. *J. Am. Chem. Soc.* 1967, **89**, 1147-1157.
44. Challis, B. C.; Shuker, D. E. G. The chemistry of nitroso-compounds. XIV. Nitrosation of amines in aqueous solution by dissolved gaseous NOCl. *J. Chem. Soc. Perkin II* 1978, 1020-1024.
45. Challis, B. C.; Shuker, D. E. G. Chemistry of nitroso-compounds. XVI. Formation of *n*-nitrosamines from dissolved NOCl in the presence of alkanolamines and related compounds. *Food Cosmet. Toxicol.* 1980, **18**, 283-288.
46. Challis, B. C.; Outram, J. R. The chemistry of nitroso-compounds. IV. Formation of *N*-nitrosamines in solution from gaseous nitric oxides in the presence of iodine. *J. Chem. Soc. Perkin I* 1979, 2768-2775.
47. Hildrum, K. I.; Williams, J. L.; Scanlon, R. A. Effect of sodium chloride concentration on the nitrosation of proline at different pH levels. *J. Agric. Food Chem.* 1975, **23**, 439-442.
48. Greenwood, D. A. Some further studies on the destruction of sodium nitrite by heating. In: *Proc. American Meat Institute*, Washington, DC, 1940, pp. 41-46.
49. Fujimaki, M.; Emi, M.; Okitani, A. Fate of nitrite in meat curing model systems composed of myoglobin, nitrite and ascorbate. *Agric. Biol. Chem.* 1975, **39**, 371-377.
50. Olsman, W. J.; Krol, B. Depletion of nitrite in heated meat products during storage. In: *18th European Meeting Meat Research Workers*, Guelph, Canada, 1972, p. 409.
51. Lee, M.; Cassens, R. G. Effect of sodium chloride on residual nitrite. *J. Food Sci.* 1980, **45**, 267-273.
52. Hildrum, K. I. Effects of pH and sodium chloride concentration on the reaction between nitrite and sulfanilic acid/*N*-(naphthyl) ethylene-diamine. *J. Assoc. Off. Analyt. Chem.* 1979, **62**, 956-958.
53. Fox, J. B., Jr. The determination of nitrite. *CRC Critical Rev. In: Anal. Chem.* 1985, **15**, 283-313.
54. Reith, J. F.; Szakaly, M. Formation and stability of nitric oxide myoglobin. I. Studies with model systems. *J. Food Sci.* 1967, **32**, 188-193.
55. Fox, J. B., Jr.; Rorer, F. P.; Fiddler, R. N.; Carroll, R. J.; Wasserman, A. E. Combined effect of nitrite and chloride on the ultrastructure of meat as shown by scanning electron microscopy. *J. Food Sci.* 1980, **45**, 1056-1059.
56. Hustad, G. O.; Cervený, J. G.; Trenk, H.; Deibel, R. H.; Kautter, D. A.; Fazio, T.; Johnston, J. W.; Kolari, O. E. Effect of sodium nitrite and sodium nitrate on botulinal toxin production and nitrosamine formation in weiners. *Appl. Microbiol.* 1973, **26**, 22-26.
57. Herring, H. K. Effect of nitrite and other factors on the physico-chemical characteristics and nitrosamine formation in bacon. In: *Proc. of the Meat Industry Research Conference*, American Meat Institute Foundation, Chicago, IL, 1973, pp. 47-60.
58. Rubin, L. J. Nitrites and nitrosamines in perspective. *Can. Inst. Food Sci. Tech.* 1977, **10**, A11-A13.
59. Lin, H. S.; Sebranek, J. G. Effect of sodium nitrite concentration and packaging conditions on color stability and rancidity development in sliced bologna. *J. Food Sci.* 1979, **44**, 1451-1454.
60. Fox, J. B., Jr.; Townsend, W. E.; Ackerman, S. A.; Swift, C. E. Cured color development during frankfurter processing. *Food Technol.* 1967, **21**, 68A-74A.
61. Mottram, D. S.; Rhodes, D. N. Nitrite and the flavour of cured meat. I. In: *International Symposium on Nitrite in Meat Products* (Krol, B.; Tinbergen, B. J., Eds), Pudoc, Wageningen, The Netherlands, 1974, pp. 161-171.

62. Ingram, M. The microbiological effects of nitrite. In: *International Symposium on Nitrite in Meat Products* (Krol, B.; Tinbergen, B. J., Eds.), Pudoc, Wageningen, The Netherlands, 1974, pp. 63–75.
63. Dethmers, A. E.; Rock, H.; Fazio, T.; Johnston, R. W. Effect of added sodium nitrite and sodium nitrate on sensory quality and nitrosamine formation in thuringer sausage. *J. Food Sci.* 1975, **40**, 491–495.
64. MacDougall, D. B.; Mottram, D. S.; Rhodes, D. N. Contribution of nitrite and nitrate to the colour and flavour of cured meats. *J. Sci. Food Agric.* 1975, **26**, 1743–1754.
65. Williams, J. C.; Greene, B. E. Plate waste of bacon cured with and without nitrite. *J. Food Sci.* 1979, **44**, 1260–1262.
66. Wasserman, A. E.; Kimoto, W. Consumer evaluation of the flavor of bacon cured with and without sodium nitrite. In: *Proc. Second International Symposium on Nitrite in Meat Products*. (Tinbergen, B. J.; Krol, B.; Eds). Pudoc, Wageningen, The Netherlands, 1977, pp. 73–74.
67. Rhee, K. S.; Terrell, R. N.; Quintanilla, M.; Vanderzant, C. Effect of addition of chloride salts on rancidity of ground pork inoculated with *Moraxella* or a *Lactobacillus* species. *J. Food Sci.* 1983, **48**, 302–303.
68. Ellis, R.; Gaddis, A. M.; Currie, G. T.; Thornton, F. E. Sodium chloride effect on autoxidation of the lard component of a gel. *J. Food Sci.* 1970, **35**, 52–56.
69. Neer, K. L.; Mandigo, R. W. Effects of salt, sodium tripolyphosphate and frozen storage time on properties of a flaked, cured pork product. *J. Food Sci.* 1977, **42**, 738–742.
70. Watts, B. M.; Peng, D. W. Rancidity development in raw versus precooked frozen pork sausage. *J. Home Econ.* 1947, **39**, 88–92.
71. Greene, B. E.; Price, L. G. Oxidation-induced color and flavor changes in meat. *J. Agric. Food Chem.* 1975, **23**, 164–167.
72. Pearson, A. M.; Love, J. D.; Shorland, F. B. Warmed over flavor in meat, poultry and fish. *Adv. Food Res.* 1977, **23**, 1–74.
73. MacDonald, B.; Gray, J. I.; Kakuda, Y.; Lee, M. L. Role of nitrite in cured meat flavor: chemical analysis. *J. Food Sci.* 1980, **45**, 889–892.
74. MacDonald, B.; Gray, J. I.; Gibbins, L. N. Role of nitrite in cured meat flavor: antioxidant role of nitrite. *J. Food Sci.* 1980, **45**, 893–897.
75. Sato, K.; Hegarty, G. R. Warmed over flavor in cooked meats. *J. Food Sci.* 1971, **36**, 1098–1102.
76. Igene, J. O.; King, J. A.; Pearson, A. M.; Gray, J. I. Influence of heme pigments, nitrite and non-heme iron on development of warmed over flavor (WOF) in cooked meat. *J. Agric. Food Chem.* 1979, **27**, 838–842.
77. Zubillaga, M. P.; Maerker, G.; Foglia, T. A. Antioxidant activity of sodium nitrite in meat. *J. Am. Oil Chem. Soc.* 1984, **61**, 722–726.
78. Kanner, J. S-nitrosocysteine (RSNO), an effective antioxidant in cured meat. *J. Am. Oil Chem. Soc.* 1979, **56**, 74–76.
79. Kanner, J.; Juven, B. J. S-nitrosocysteine as an antioxidant, color developing and anticlostridial agent in comminuted turkey meat. *J. Food Sci.* 1980, **45**, 1105–1112.
80. Dennis, M. J.; Davies, R.; McWeeny, D. J. The transnitrosation of secondary amines by S-nitrosocysteine in relation to N-nitrosamine formation in cured meats. *J. Sci. Food Agric.* 1979, **30**, 639–645.
81. Dennis, M. J.; Massey, R. C.; McWeeny, D. J. The transnitrosation of N-methylaniline by a protein bound nitrite model system in relation to N-nitrosamine formation in cured meats. *J. Sci. Food Agric.* 1980, **31**, 1195–1200.
82. Challis, B. C.; Osborne, M. R. The chemistry of nitrosocompounds. VI. Direct and indirect transnitrosation reactions of N-nitrosodiphenylamine. *J. Chem. Soc. Perkin II* 1973, 1526–1533.
83. Benedict, R. C. Biochemical basis for nitrite inhibition of *Clostridium botulinum* in cured meat. *J. Food Protect.* 1980, **43**, 877–891.
84. Sofos, J. N.; Busta, F. F.; Allen, C. E. Botulism control by nitrite and sorbate in cured meats: a review. *J. Food Protect.* 1979, **42**, 739–770.
85. Tompkin, R. B.; Christiansen, L. N.; Sheparis, A. B. Cause of variation in botulinal inhibition in perishable canned cured meat. *Appl. Environ. Microbiol.* 1978, **35**, 886–889.
86. Tompkin, R. B.; Christiansen, L. N.; Sheparis, A. B. The effect of iron on botulinal inhibition in perishable canned cured meat. *J. Food Technol.* 1978, **13**, 521–527.
87. Tompkin, R. B.; Christiansen, L. N.; Sheparis, A. B. Iron and the antibotulinal efficacy of nitrite. *Appl. Environ. Microbiol.* 1979, **37**, 351–353.
88. Tompkin, R. B. The role and mechanism of the inhibition of *C. botulinum* by nitrite—is a replacement available? In: *Proc. of the Reciprocal Meat Conference*, Storrs, CT. American Meat Science Association, 1978, pp. 135–147.
89. Woods, L. F. J.; Wood, J. M.; Gibbs, P. A. The involvement of nitric oxide in the inhibition of the phosphoroclastic system in *Clostridium sporogenes* by sodium nitrite. *J. Gen. Microbiol.* 1981, **125**, 399–406.
90. Reddy, D.; Lancaster, J. R., Jr.; Cornforth, D. P. Nitrite inhibition of *Clostridium botulinum*: electron spin resonance detection of iron-nitric oxide complexes. *Science* 1983, **221**, 769–770.
91. Tompkin, R. B.; Christiansen, L. N.; Sheparis, A. B. Enhancing nitrite inhibition of *Clostridium botulinum* with iso-ascorbate in perishable canned cured meat. *Appl. Environ. Microbiol.* 1978, **35**, 59–61.
92. Tompkin, R. B.; Christiansen, L. N.; Sheparis, A. B. Antibotulinal role of isoascorbate in cured meat. *J. Food Sci.* 1978, **43**, 1368–1370.
93. Perigo, J. A.; Roberts, T. A. Inhibition of clostridia by nitrite. *J. Food Technol.* 1968, **3**, 91–94.
94. O'Leary, V. S.; Solberg, M. Effect of sodium nitrite inhibition on intracellular thiol groups and on the activity of certain glycolytic enzymes in *Clostridium perfringens*. *Appl. Environ. Microbiol.* 1976, **31**, 208–212.

95. Buchanan, R. L., Jr.; Solberg, M. Influence of sodium nitrite on the aerobic catabolism of glucose by *Staphylococcus aureus*. *J. Food Safety* 1978, **1**, 189-200.
96. Lee, S. H.; Cassens, R. G.; Sugiyama, H. Factors affecting inhibition of *Clostridium botulinum* in cured meats. *J. Food Sci.* 1978, **43**, 1371-1374.
97. Wasserman, A. E.; Huhtanen, C. N. Nitrosamines and the inhibition of clostridia in medium heated with sodium nitrite. *J. Food Sci.* 1972, **37**, 785-786.
98. Freese, E.; Sheu, C. W.; Galliers, E. Function of lipophilic acids as antimicrobial food additives. *Nature* 1973, **241**, 321-323.
99. Roberts, T. A.; Ingram, M. Inhibition of growth of *Clostridium botulinum* at different pH values by sodium chloride and sodium nitrite. *J. Food Technol.* 1973, **8**, 467-475.
100. Roberts, T. A.; Gibson, A. M.; Robinson, A. Prediction of toxin production of *Clostridium botulinum* in pasteurized pork slurry. *J. Food Technol.* 1981, **16**, 337-355.
101. Robinson, A.; Gibson, A. M.; Roberts, T. A. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats. V. Prediction of toxin production: Non-linear effects of storage temperature and salt concentration. *J. Food Technol.* 1982, **17**, 727-744.